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T-Cell Signaling in HIV-1 Infection

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Abstract: HIV exploits the T-cell signaling network to gain access to downstream cellular components, which serves as effective tools to break the cellular barriers. Multiple host factors and their interaction with viral proteins contribute to the complexity of HIV-1 pathogenesis and disease progression. HIV-1 proteins gp120, Nef, Tat and Vpr alter the T-cell signaling pathways by activating multiple transcription factors including NF-κB, Sp1 and AP-1. HIV-1 evades the immune system by developing a multi-pronged strategy. Additionally, HIV-1 encoded proteins influence the apoptosis in the host cell favoring or blocking T-cell apoptosis. Thus, T-cell signaling hijacked by viral proteins accounts for both viral persistence and immune suppression during HIV-1 infection. Here, we summarize past and present studies on HIV-1 T-cell signaling with special focus on the possible role of T cells in facilitating viral infection and pathogenesis.

Keywords: Apoptosis, HIV-1, Nef, reservoirs, T cells, viral proteins.

INTRODUCTION

The ineffective cell-mediated and humoral response to HIV-1 infection results in partial control of viral replication that ultimately leads to chronic immune activation and systemic depletion of CD4+ T cells [1]. The long period of time between infection and development of AIDS reveals that only a small fraction of CD4+ T cells which are infected during acute HIV-1 infection, elevates the death rates of T cells through direct or indirect (bystander T- cell apoptosis) manner, and are responsible for HIV disease progression [2]. Immune system struggles to recover the injury made by HIV during acute HIV-1 infection but HIV-1 develops highly effective strategies to overcome host immune system [3]. This struggle is further complicated by opportunistic infections [4]. This battle causes further damage to the immune system by providing fuel for viral replication that ultimately leads to the formation of viral reservoirs [5].

The primary infection is defined as the first period of infection from onset of disease until the formation of HIV-1 specific antibodies 3 to 4 weeks of infection [6]. During HIV transmission, dendritic cells (DCs) are first line of defense against viral penetration and wide spread dissemination [7]. DCs take up HIV *via* C-type lectin binding receptor and migrate to lymph nodes where they prime HIV-specific immune responses by presenting HIV-1 antigens to CD4+ T cells [8]. However, it has been recently reported that Siglec-1 is a key factor for HIV-1 spread via infectious DC/T-cell synapses [9-10]. Furthermore, it is likely that DCs contribute to HIV-1 dissemination throughout the body during early stages of infection due to their migratory potential [11]. Moreover, CD4+ T cells help CD8+ T cells and B cells in mounting cellular and humoral anti-HIV immune response. The plasma viremia increases to reach a peak after 21-28 days of infection together with depressed peripheral CD4+ T cell numbers [12]. This acute HIV-1 infection results in selective and dramatic depletion of CD4+ CCR5+ memory T cells predominantly at mucosal surfaces that have severe immunological consequences. During early SIV infection, up to 60% of memory CD4+ T cells in the intestinal lumina propria appear to contain SIV-RNA at the peak of infection at day 10, with majority of these cells being eliminated by day 14 [12]. These results further characterize the early loss of memory CD4+ T cells in the mucosal associated lymphoid tissue (MALT), which follows SIV infection. Additionally, the infection of CD4+ T cells that express low level of CCR5 emphasizes the infection of resting memory CD4+ T cells and the loss of uninfected CD4+ T cells as a further mechanism of early CD4+ T cell depletion in the MALT [12, 13].

At the peak of viremia, patients may develop symptoms of acute retroviral syndrome e.g., influenza like illness with fever, sore throat, lymphadenopathy and exanthema [14]. However, viral reservoirs have already been established in cells with slower rate of decay than T cells, implying that virus cannot be eliminated by highly antiretroviral therapy (HAART) within the lifetime of the patient [15]. Immunological damages to gastrointestinal tract lead to breaks in the mucosal barrier allowing the translocation of microbial products into circulation that is responsible of chronic immune activation [16]. In this scenario, the ultimate consequence of immune activation is depletion of CD4+ and CD8+ T cells, abnormal T-cell trafficking, clonal exhausting of T cells and drainage of memory T-cell pools [17]. In addition, the accelerated viral evolution at this stage provided by an excessively high viral mutation rate and alternation in cellular tropism, resulting in progression from a pool of CCR5 tropic to dual tropic or dominantly CXCR4 tropic strains with increase virulence and broader target cell tropism [17]. Damage to lymphoid tissue results in thymic

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cellular proteins and signaling complexes in a timely and well defined manner [25]. Nef down-regulates the cell surface receptors CD4 [26], CD28 [27], CD3 (only in SIV) [28] and major histocompatibility complex (MHC) class I, A and B [29] and upregulates FasL [30-32]. In addition to its expression in virally infected cells, Nef is also present in the extracellular environment and can reach concentration of up to 10 ng/ml in the sera of HIV-1-infected individuals [33]. This concentration may be higher in lymph nodes where virion trapping dendritic cells as well as virion infected CD4+ T cells and antigen-presenting cells (APC), are tightly packed [34, 35]. Infected cells may release Nef through non-classical secretory pathway or after lysis. Moreover, bystander cells may internalize Nef *via* endocytosis, pinocyotsis or other yet undefined mechanisms. However, it has been reported that HIV-1 Nef protein modulates the expression of a significant number of genes as early as two hours after treatment [36].

HIV-1 Nef protein activates T-cell signaling pathway and was originally described in 1994 using Jurkat cells stably transfected with CD8-Nef chimeric protein [37]. Cells that had higher concentration of CD8-Nef at the plasma membrane induced tyrosine phosphorylation and upregulation of activation markers such as CD69 [37]. In addition, activated Jurkat T cells die by activation induced apoptosis, and only cells with mutated *nef* genes expressing truncated Nef survived, indirectly confirming that Nef activated these cells [37]. Additionally thymocytes of transgenics expressing Nef under control of CD4 promoter exhibit constitutive tyrosine phosphorylation of LAT and p42/44 MAP kinase and CD3 hyperactivity [38]. Moreover, NFAT activity is increased in highly pathogenic strain of SIV, SIVmac239, containing a Nef variant with a functional immunoreceptor tyrosine-based activation motif (ITAM) [39]. The impact of Nef on T-cell activation was further investigated in which it was demonstrated that Nef associates with membrane microdomains critically involved in the initiation and propagation of T-cell signaling. This raft association was required for Nef-mediated activation of NF-κB, NFAT, IL-2 and HIV-1 long terminal repeat (LTR) stimulation following CD3/CD28 costimulation [40, 41]. These results were further confirmed by gene expression profiling of inducible T-cell lines, showing that Nef and anti-CD3 mediated T-cell activation overlaps by 97% [42]. Moreover, it has been recently reported that HIV-1 Nef modulated the TCR functions either positively or negatively depending upon the activation state of infected T cells [43].

The direct interaction of Nef with both the T-cell receptor and its immediate downstream effectors has been reported [44]. Functional as well as binding studies analyzed the interaction of Nef with the T-cell receptor-chain [45], and proteins of the T-cell environment, like adaptor protein Vav [46] and LAT [38], the tyrosine kinase Lck [47], the serine kinase Pak [48], PKC [49], the DOCK2-ELMO1 complex [50], the map kinase ERK1 and ERK2 [51], and membrane microdomains [40]. Nef is a modular protein containing a myristoylated N-terminus, a core domain and a polyproline motif. The polyproline motif acts as an SH3 binding domain that is highly conserved in viral isolates. This domain is capable of mediating association with Src kinase [52], and alters the catalytic activity of different kinases such as Lck and Hck [47-53]. Furthermore it also facilitates Nef binding to Vav and Vav2, which results in cytoskeletal changes and activation of JNK signaling pathways [46]. In addition, the polyproline motif has been shown to regulate the interaction with TCR ζ-zeta chain [45-54]. The core domain of HIV-1 Nef protein contains di-arginine motif that allows the functional interaction with PAK kinase associated with increased viral infectivity [55, 56].

Nef mediated T-cell activation seems completely fulfill the needs of HIV, as one of the very early finding was that T cells have to be activated for HIV-1 replication and infection to start [57]. As quiescent T cells do not support efficient retrotranscription, integration, expression of HIV genome due to low level of nucleotides, ATP and nuclear transcription factors [58, 59]. Nef mediated T-cell activation leads to nuclear translocation of transcription factor such as NFAT and NF-κB, activating the viral promoter or establishing a basal viral transcription that would leads to the expression of more Tat protein [60]. However, HIV does express Nef before virus integration [23], but the viral replication in resting CD4 T cells is very low. The stimulation of TCR by antibodies activates viral replication. Although, Nef alone may not result in optimal viral replication [61], it may do with some additional cellular support that comes from macrophages or DCs. In immature DCs, HIV-1 replicates at a very low level. However, upon co-culture with resting T cells, a significant viral replication is observed in the T cells that require a functional nef gene [62, 63]. In addition, macrophages play a supporting role for HIV-1 replication. Infected macrophages secrete chemokines (MIP-1α and MIP-1β) in a Nef-dependent manner. The released chemokines attract the resting T cells and stimulate them for productive infection [64, 65]. Moreover, immature DCs also attract T cells in a Nef-dependent manner by upregulating DC-SIGN [66].

Survival strategies are more important for invading pathogenic viruses, in particular when they establish a chronic infection [32]. HIV-1 through Nef interferes with MHC molecules, modulates cytokine activity, and induces apoptosis by FasL [32, 67]. In HIV-infected cells, the Nef expression leads to the upregulation of FasL, which could potentially stimulate the Fas receptor in an auto/paracrine fashion. Such a mechanism leads to the destruction of infected cells that overexpress Fas [44, 68]. Additionally, HIV-1 gp120 ligation of CXCR4 on macrophages induces upregulation of membrane bound TNF, triggering cell death *via* TNFR in adjacent CD8+ T cells which leads to CD8+ T cell depletion [69].

HIV-1 Nef manages the apoptotic signal from cell surface receptors through association and blockade of apoptosis signal regulating kinase 1 (ASK1) [70]. ASK1 links both the Fas and TNFR mediated signals to downstream JNK/p38 pathways. ASK1 kinase activity is inhibited by thioredoxin (Trx), a redox regulator protein. Only a reduced form of Trx associates with ASK1 and keeps the kinase inactive [71]. HIV-1 Nef targets the ASK1 by blocking the release of Trx from ASK1 [70]. Nef signaling also interferes with the regulation of intrinsic cellular death pathway by Bcl family of proteins. HIV-1 Nef associates with and activates PI3K but not to stimulate Akt, but, rather to activate Nef-associated serine kinase Pak [72]. The Nef-PI3K-PAK complex phosphorylates Bad on serine residues,

resulting in block of apoptosis induced by serum starvation and, more importantly, by HIV replication [72]. In this way, Nef manages the difficult task like keeping the cell alive until next generation of virus is ready to strike.

HIV-1 gp120

The gp120 molecule of HIV-1 is a glycoprotein, essential for viral infection as it facilitates HIV entry into the host cells [73]. HIV-1 gp120 is shed from the viral membrane and accumulates in lymphoid tissues in significant amounts [74], e.g. where it can induce apoptosis and severely alter the immune response to the virus by dampening the antiviral CTL response thus impeding the clearance of HIV [75]. Binding of HIV envelope to its chemokine coreceptors (CXCR4, CCR5) mediates two major biological functions: membrane fusion and signal transduction. Apart its function in facilitating viral entry, it is becoming increasingly evident that gp120 plays a much greater role in HIV pathogenesis [76]. The binding of viral envelope to its chemokine receptors, CXCR4 and CCR5, not only mediates entry but also activates multiple intracellular signaling cascades, a process mimicking chemokine signaling through binding to their cognate receptors [77]. The coreceptor CCR5 is used during early infection whereas the virus utilizes the CXCR4 coreceptor during later infection in 40% of patients [78, 79]. The X4 and R5 envelopes (expressed on infected cells] can induce rapid tyrosine phosphorylation of the protein tyrosine kinase Pyk2 through binding to CXCR4 or CCR5 [80]. Pyk2 phosphorylation is frequently associated with G protein signaling and calcium flux [81]. Chemokine receptor signaling is coupled with distinct pathways that mediate cell migration, transcriptional activation, cell growth and differentiation. CXCR4 activates phospholipase C-γ (PLC-γ) that leads to calcium flux and activation of protein kinase C (PKC). PKC signaling is important for SDF-α chemotaxis. During viral entry, HIV-1 gp120 binding to CXCR4 or CCR5 activates a number of signaling molecules common to chemokine mediating signaling. In addition to Pyk2 activation, gp120 triggers the activation of PI3K, Akt [82, 83], Erk-1/2 [83] and CD4/CXCR4 dependent NFAT nuclear translocation [84].

HIV-1 gp120 mediates chemotaxis, actin cytoskeleton rearrangement and the activation of an actin depolarization factor, cofilin, to increase the cortical actin dynamics in resting CD4+ T cells [77]. Moreover, recombinant viral envelope induces viral replication in culture of resting of CD4+ T cells of infected patients [85]. Furthermore, the HIV envelope enhances the viral infectivity by facilitating the nuclear import of pre-integration complex [86, 87]. Cofilin is involved in chemotaxis and T-cell activation [88]. In chemotaxis, cofilin is the main driving force for promoting the cortical actin dynamics central to cell migration [89]. In T-cell activation, cofilin is activated through CD28 co-stimulation, and plays a critical role in actin reorganization and formation of immunological synapse required for sustained T-cell activation [90]. The static cortical actin in resting CD4+ T cells is a unique barrier for viral post entry migration [91]. To overcome this restriction, HIV relies on gp120-CXCR4 signaling and activates cofilin to increase the cortical actin dynamics. This unique requirement is observed in resting CD4+ T cells, since in activated T cells cofilin is constitutively active to facilitate the cell cycle driven actin remodeling [91].

Significant amounts of soluble gp120 are present in lymph nodes during chronic HIV infection [75]. T cells from these areas lose their ability to respond to gp120. Moreover, the regulatory T cells produce immunosuppressive cytokines such as TGF-β upon stimulation of SHIV gp120 [75, 92]. Much of immunosuppressive activity of gp120 is mediated by its heavily glycosylated variable loops which contain mannose residues that inhibit the T-cell proliferation by inhibiting the ability of mature monocyte derived dendritic cells (mMDDC) to induce T-cell proliferation [93]. Accordingly, enzymatic removal of mannose moieties increases immune responses to gp120 [94].

Tat

HIV-1 Tat is virally encoded multifunctional protein, which plays a critical role in viral replication [95]. Tat is an early regulatory protein that has variable length of 86-104aa, encoded by two exons [96]. The first encodes residues 1 to 72 and is classically described as a modular protein, which is sufficient for Tat transactivation [95]. The second exon of Tat codes for amino acid 73-104, essential for NF-kB dependent HIV-1 LTR activation [97]. Mutational analysis of HIV-1 Tat has found two functional domains: an activation domain that mediates its interaction with cellular machinery and an arginine rich region that is required for binding to the transactivation responsive element (TAR) RNA [98]. The laboratory HIV-1 strains produce an active 86 aa Tat protein whereas most of Tat proteins from primary isolates contain an additional sequence at their C-terminus. The soluble form of Tat, which is released from productively infected unruptured cells as an extracellular protein and in the serum of HIV-1 infected individuals, is also able to enter in neighboring cells [99, 100]. Specific Tat binding has been reported for three cell surface receptors including heparin sulphate, beta integrin and chemokine receptors. Studies of Tat derived peptides have shown that residues 48-60 from the protein transduction domain (PTD) account for the functional internalization into cells [101-103]. HIV-1 Tat is phosphorylated by CDK2 *in vitro* and *in vivo*. The Ser16 and Ser46 residues of Tat are potential phosphorylation sites. The phosphorylation of Tat is critical for HIV-1 transcription [104, 105].

HIV-1 Tat modulates the expression of several cellular genes [106]. Tat has been shown to upregulate the expression of IL-10 [107]. Furthermore, *in vivo* studies indicate that there is increase in IL-10 production in HIV-1 infected patients [108]. IL-10 inhibits the synthesis of TH1 and pro-inflammatory cytokines such as IFN-ɣ, IL-2, IL-3, TNF-α and GM-CSF [109, 110]. It has been shown HIV-1 Tat protein suppresses gp-120 specific T-cell response in IL-10 dependent fashion. This immunosuppressive effect of Tat is not observed in IL-10 deficient mice demonstrating that the immunosuppressive effect of Tat is mediated through IL-10 [107]. Additionally, HIV-1 utilizes the Tat protein to hijack the intracellular functions and evades the immune response of the host. It has been shown that secreted form of Tat from infected cells induces the expression of specific chemokine receptors such as CCR5 and CXCR4, which are important for HIV-1 infection [111]. Furthermore HIV-1 Tat

activates uninfected naïve T cells (independent of antigen stimulation) and favors productive infection [112-114]. HIV-1 Tat provides a reliable way for virus to compensate for the rapid destruction of activated permissive T cells during the highly cytopathic infection. The soluble Tat protein enters into T cells and interferes with IL-7 signaling by down-regulating the IL-7 receptor (CD127) [115]. IL-7 is essential for T-cell development and for maintaining homeostasis of mature T cells [116]. Furthermore, it has been reported that CD127 is downregulated on the surface of CD8+ T cells isolated from HIV-infected patients [117, 118]. Moreover, the treatment of recombinant, fully glycosylated simian IL-7 prevents the decline of circulating CD4+ T cells during acute phase of SIV infection in rhesus macaques [119]. HIV-1 Tat protein activates the MAPK pathways in primary T cells which is associated with the progression from G0 to G1 phase in naïve T cells facilitating productive HIV infection [112]. HIV-1 Tat upregulates the IL-2 production with CD3 or CD28 costimulation in T cells [120]. Moreover, HIV-1 Tat mediates the CD4+ T cells loss by recruiting the quiescent T cells in a reservoir that is permissive to productive HIV-1 infection and destruction by virus, and also induces apoptosis in uninfected T cells [112, 121]. Finally HIV-1 Tat down-regulates the expression of several genes like the gene encoding major histocomptability (MHC) class I [122].

Tat has been shown to induce apoptosis of bystander CD4+ T cells by upregulating Fas ligand expression in both infected and uninfected bystander cells [123]. The T cells (CD4+ and CD8+ T cells) in HIV-1 infected individuals are more susceptible to Fas ligand induced apoptosis, as infected CD4+ T cells overexpress Fas and the proportions of these CD4+ T cells increase with the disease progression [124]. Therefore, the upregulation of FasL by Tat may lead to increased apoptosis in the antigen responding T cells that are overexpressing Fas [124, 125]. HIV-1 Tat protein associates and increases the transcription of phosphatase, PTEN and PP2A. The upregulation of these proteins in HIV-1 infected CD4+ T cells results in decreased amounts of pAkt and increased amounts of non-phosphorylated FOXO3, which activates the transcription of its proapoptotic target genes [126].

HIV-1 infection results in increased production of inflammatory cytokines (TNF-α, IL-1, IL-6) that enhances viral gene expression and viral replication, and finally fuels virus spread [127-129]. The first exon of HIV-1 Tat increases gene expression of TNF by activating the TNF promoter [130]. Furthermore, T cells chronically infected with a pol-defective HIV-1 provirus constitutively express significantly higher levels of Tat and TNF [130]. Since TNF enhances the production of proinflammatory cytokines (IL-1 and IL-6) that ultimately enhances HIV-1 gene expression [128], thereby HIV-1 uses Tat protein and host factors to increase its own expression and infectivity to fuel disease [129].

IL-2 plays a very significant role in immune response and is regarded as a T-cell growth factor [131]. IL-2 has direct effects on the development and proliferation of lymphocytes, monocytes, macrophages and oligodendrocytes [131]. HIV-1 Tat protein upregulates the IL-2 gene expression and IL-2 secretion in Jurkat T cells and in primary T cells [132]. T-cell activation is known to be required for efficient viral replication and propagation [112]. Therefore, enhanced IL-2 secretion might enhance viral replication and spread [132].

Vpr

Viral protein R (Vpr), a 12-15-kDa virion-associated protein, is composed of 96 amino acids and is highly conserved in HIV-1 and SIV [133-135]. Vpr performs several functions such as nuclear import of viral preintegration complex, induction of G2 cell cycle arrest, modulation of T-cell apoptosis, transcriptional coactivation of viral and host genes, and regulation of NF-κB activity [136]. Vpr is an essential HIV-1 protein for efficient infection in non-dividing cells [137] and enhances HIV-1 replication in T-cell lines and activated peripheral blood lymphocytes *in vivo* [138]. Recombinant Vpr stimulates the HIV-1 transcription from LTR and the formation of ion selective channels in lipid bilayers [139, 140].

Vpr protein is present in significant amount in the serum of AIDS patients [141]. Initially, it was observed that Vpr reactivates HIV-1 from latently infected cell lines and peripheral blood mononuclear cells of HIV-1 infected individuals [141, 142]. Moreover, later studies demonstrated that Vpr activates HIV-1 LTR as well as other cellular promoters [139, 143, 144]. The U3 region of HIV-1 LTR contains several activating elements such as NFAT, GRE, NRF, NF-κB and Sp1 [145-147]. Vpr transactivates the HIV-1 LTR through the interaction with the cellular transcription factor Sp1 [144]. Sp1 is ubiquitously expressed and is involved in the transcription of a variety of cellular genes [148, 149]. However, other studies support the findings, that Vpr transactivates primarily the -278 to -176 region of LTR, which contains GREs, while the NF-kB and Sp1 are utilized by Tat mediated transactivation [150]. The Vpr mediated HIV-1 LTR transactivation plays an important role for immediate early expression of HIV-1 genome when alternative positive regulators are low [144]. HIV-1 Vpr binds to the transcription factor TFIIB and Vpr acts as a co-activator for transcription [143]. HIV-1 Vpr potentiates HIV-1 LTR activation by forming a complex with p300 and TFIIH [151, 152]. Additionally, Vpr has also been reported to act cooperatively with HIV-1 Tat [153]. Therefore, the production of viral particle is likely increased *via* coactivation of HIV-1 LTR by Vpr. Moreover, Vpr binds to GR and activates GRE which in turn regulates the transcription of cellular genes which may increase HIV-1 replication and permissiveness [154].

Expansion of HIV-1 specific CD4+ T cells results in effective maintenance of immune system and contributes to control of viremia [155, 156]. The presence of virus specific CD8+ T cell response is essential for viral clearance during HIV-1 infection [155, 157]. Additionally, CD8+ T cell response can inhibit HIV-1 replication *in vitro* and also control the viral load in HIV-1 infected patients [158]. The loss in number of effective CD8+ T cells in HIV-1 infected patients has been correlated with reduced antiviral effects and disease progression in parallel with deterioration of immune system [159, 160]. It has been reported that HIV-1 Vpr interferes with the development of antigen specific immunity [161]. It specifically inhibits the development of strong CD8+ CTL response and suppresses the Th1 immune

responses by down-regulating IFN-ɣ production. In the presence of Vpr, there is an isotype shift towards Th2 response [161]. Moreover, Vpr reduces the efficacy of DNA and SIV-Nef vaccination *in vivo*, suggesting that Vpr may aid in evasion of immune response during HIV-1 [161, 162]. The mechanism of immune dysfunction caused by Vpr appears to involve the induction of apoptosis and cell cycle arrest in bystander T cells, contributing to the depletion of immune cells. While Vpr is seemingly anti-apoptotic in HIV-1 infected cell lines, however, *in vitro* studies suggest that bystander T cells may be induced to undergo apoptosis in response to extracellular or soluble Vpr [163-165]. Vpr alone has been shown to contribute to HIV-1 mediated immune dysfunction by promoting the depletion of thymic cells [166]. In addition to activation induced cell death by apoptosis of CD4+ T cells, Vpr induces apoptosis by multiple mechanisms [167, 168]. Vpr increases Fas dependent caspase 8 activation in T cells to induce apoptosis, providing a potential mechanism for increased cell death. CD4 promoter in Vpr transgenic mice shows T-cell depletion in a Bcl-xL, Bax and caspase 1 dependent manner [169]. Vpr induces G2 cell cycle arrest that is associated with cell death [170]. This property depends on Vpr activated phosphorylation of Chk1, an event that begins during S phase of cell cycle [171]. Apoptosis occurs *via* caspase-9 and also causes apoptosis in cancer cell lines with mutated p53, suggesting this function is independent of p53 function [172, 173]. Furthermore, Vpr has been reported to increase the expression of TNF-α on dendritic cells and thereby could favor the apoptosis of CD8+ T cells [174]. The Vpr-mediated depletion of bystander T cells likely contributes, in part, to immune dysfunction observed in AIDS. Additional mechanism of Vpr mediated T-cell death has been reported. Vpr alone is sufficient to upregulate NKG2D ligand expression in CD4+ T lymphocytes, which results in NK mediated cell death [175, 176]. Finally Vpr has also been reported to inhibit the NK activity [174, 177].

Vpr suppresses the cellular immunity by antigen-mediated activation and cytotoxic killing of surviving T cells. *In vivo*, it suppresses the Th1 cytokines (IFN-ɣ, IL-12) [162] and promotes the shift toward Th-2 response [178]. Vpr alters various immunoregulatory molecules at multiple levels in infected T lymphocytes to escape the host immune response. It downregulates the expression of CD28 and increased the expression of CTLA-4 [179]. CD28 and CTLA-4 are the main costimulatory molecules in T cells that interact with CD80 and CD86 on the antigen presenting cells and initiate the proliferation, differentiation and effector functions. HIV-1 Vpr differentially regulates the expression of cell surface molecules and impaired IFN-ɣ production that is involved in T-cell activation [179]. Vpr has also been shown to suppress the immune activation to superantigens *in vivo* [180]. Moreover, Vpr has been shown to modulate NK cell functions, causing a reduction in cytolytic killing and differential regulation of IL-12 and TGF-β by smad-3 activation [181]. Therefore, Vpr may significantly contribute to the immune deficiency seen in AIDS by altering both adaptive and innate immune cellular functions.

The effect of Vpr on the immune system seems to be mediated by the interaction with NF-κB signaling pathway. Vpr along with GR have immunosuppressive effects due to NF-κB inhibition and induction of IκBα which prevents the NF-κB translocation into nucleus thereby preventing cytokine release and immune activation [182, 183]. Vpr induces T-cell apoptosis in a TCR-dependent mechanism by inducing IκB and inhibiting NF-κB activity [184]. Vpr down-regulates the NF-κB inducible kinase (NIK) and cytokines such as IL-2, IL-12, TNF-α, IL-4, MIP-1α, MIP-1β and RANTES [184, 185]. Moreover, these effects were reversed with RU486 treatment, suggesting that the inhibition of NF-κB *via* IκBα involves GR signaling pathway, indicating the cooperative role of Vpr and GR in suppressing the NF-κB dependent transcription activity [186]. However, several reports indicate that Vpr can also activate NF-κB signaling by inducing IκB phosphorylation and subsequent degradation [187-189].

T-CELL SIGNALING AND APOPTOSIS

T-Cell Apoptosis

Programmed cell death and necrosis are two main mechanisms by which cells die. Necrosis results from a severe cellular insult and apoptosis is controlled process that occurs without inflammation or injury to surrounding tissue. Deregulation of apoptosis can disrupt the balance between proliferation and cell death [190]. Immune system has its fundamental property to expand rapidly the antigen specific lymphocytes to combat pathogens [191]. The immune response is a multiple step process: naïve T cells are activated through cross-linking of antigen to TCR, leading to proliferation and differentiation into effector cells. The apoptosis is a crucial step for the termination of acquired immune response and the apoptotic process of elimination of activated T cells during the termination phase of an immune response is called activation induced cell death (AICD) [192-193]. HIV-1 interferes with the death pathway, and HIV-1 players that modulate apoptosis are gp120, Nef, Tat and Vpr [194] (Fig. **2**).

Direct Killing of Infected CD4+ T Cells

Loss of CD4+ T cells and immune activation are the hallmark of HIV-1 infection. The acute infection is associated with loss of CD4+ T cells and a chronic phase characterized by an immune activation with massive production of proinflammatory cytokines [195, 196] and gradual loss of peripheral CD4+ T cells [197]. During primary infection, before the onset of antiviral immune response, the number of CD4+ T cells decline in association with high viremia. Moreover, HIV-1 infects preferentially those CD4+ T cells that are HIV-1 specific and more sensitive to apoptosis [22, 198]. Loss of infected CD4+ T cells is the result of several mechanisms such as induction of syncytium formation, alteration of membrane permeability, mitochondrial dysfunction, killing by HIV-1 specific cytotoxic T cells or through the expression of death receptor due to high levels of immune activation [12]. Apoptosis by direct cytopathic effects of HIV-1 occurs *via* virus induced cytolysis and killing of virus infected cells which occurs *via* the immune surveillance through the action of cytotoxic T cells [199, 200]. Syncytia are frequently observed *in vitro* [201]. Syncytia are generated by the fusion of gp120/gp41 on the plasma membrane of HIV-1 infected cells with uninfected cells bearing a coreceptor, especially CXCR4 [202]. Syncytia are condemned to die by apoptosis due to genomic instability, and p53 seems to play a critical role in

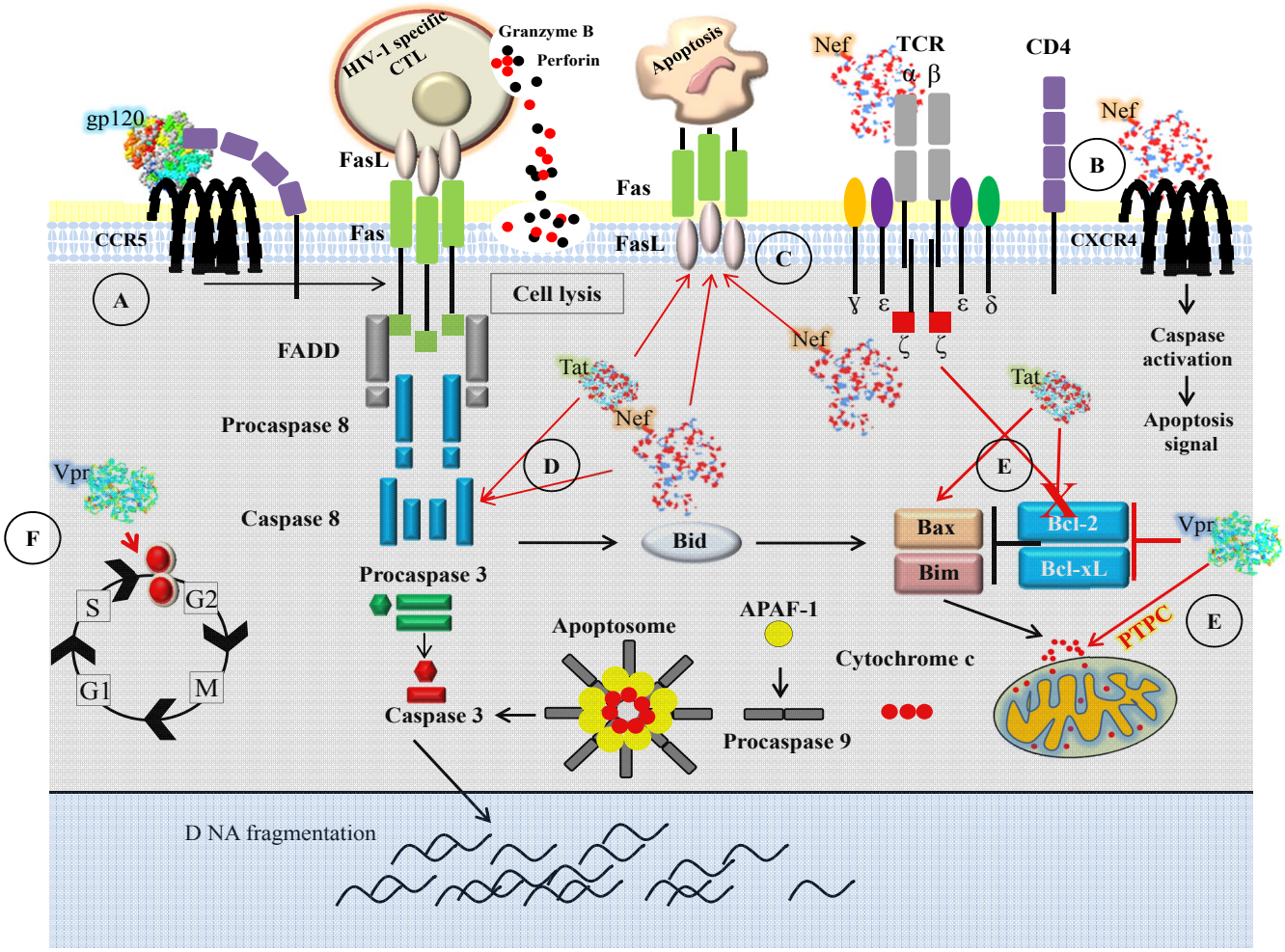

Fig. (2). T-cell death during HIV-1 infection. A) Death associated with gp120 binding to CD4 or CCR5. After binding of gp120 to CD4 or CCR5, it activates intracellular signaling pathways that increase CD4+ T-cell susceptibility to Fas-mediated apoptosis. **B**) Extracellular Nef interacts with CXCR4 surface receptors and induces apoptosis. **C**) Nef directly stimulates TCR-CD3 complex and upregulates Fas and FasL expression while also inhibiting the antiapoptotic proteins Bcl-2 family. **D**) Endogenous Nef and Tat upregulate Fas/FasL and induces apoptosis through caspase-8 pathway. **E**) Tat and Vpr inhibit Bcl-2 and Bcl-xL, and increase mitochondria dysfunctions. Vpr targets the mitochondrial permeability transition pore complex (PTPC) and causes loss of membrane potential, release of cytochrome c, and the activation of caspase-9 and caspase-3. **F**) Vpr arrests cells at the G2 phase of the cell cycle that results in apoptosis.

syncytial apoptosis [203]. However, the overall extent of cell-to-cell fusion *in vivo* has not been estimated [201].

Bystander Immune Activation and Apoptosis

HIV-1, through the induction of immune activation, generates its own targets for efficient replication. In untreated HIV-1 infection, every arm of immune system is in the state of hyperactivation such as high T-cell turnover, nonspecific T-cell activation and proliferation, polyclonal activation of B-cells and elevated levels of proinflammatory cytokines [204]. A direct contact is required between Env and CXCR4 for the bystander apoptosis of CD4+ T cells. However, soluble secreted factors from CD4+ T cells are required for the bystander apoptosis of CD8+ T cells [205]. Furthermore, it has been reported that the vast majority of bystander T-cell death in lymphoid tissue is due to abortive HIV-1 infection. As naïve CD4+ T cells are refractory to productive HIV-1 infection, after viral entry, infection is aborted as reverse transcription initiated but fails to reach completion [206, 207]. Accumulation of incomplete reverse transcription in resting CD4+ T cells activates a host defense mechanism that elicits pro-apoptotic and pro-inflammatory responses through caspase-3 and caspase-1 activation [208]. The death of activated lymphocytes serves to limit the immune response by killing the cells those are no longer needed [193]. These signaling mechanisms depend on the expression of members of TNF/TNFR superfamily such as Fas/FasL and TRAIL/DR5 (Fig. **2**) [209, 210]. Naïve T cells express little or no cell surface FasL while its expression increases during T-cell activation and undergo AICD more readily. HIV-1-infected cells are more resistant to apoptosis than uninfected cells [211]. In this way, indirect killing of T cells *via* Fas/FasL will destroy activated but uninfected cells while sparing the fraction of infected cells.

Bystander T cells undergo apoptosis upon interaction with HIV-1 infected cells and macrophages plays a major role in this process [212]. HIV-1 infected macrophages expresses apoptosis inducing ligand and induces the

apoptosis in bystander CD4+ T cells [213]. Furthermore, HIV-1 soluble envelope glycoproteins, resulting from shedding from the surface of viral particles or infected cells, have been implicated as a major cause of bystander cell death in T cells [214]. Cross-linking of cellular receptor CD4 and coreceptor CCR5 with gp120 activates Fas/FasL signaling pathway and induces apoptosis in uninfected CD4+ T cells [215]. Additionally, HIV-1 Nef induces apoptosis in bystander T cells indirectly *via* the increased expression of FasL in infected cells [45] or directly by interacting with CXCR4 chemokine receptor [216]. In addition, the secreted form of HIV-1 Tat protein enhances the susceptibility of bystander T cells to Fas mediated killing [121, 217].

The apoptosis of CD8+ T cells has been widely considered as a strategy used by HIV to evade the immune system [218]. CD8+ T cells eliminate infected cells and HIV-1 specific CD8+ T cells are highly sensitive to apoptosis [219]. It is apparent that multiple mechanisms that potentially crosstalk are involved in CD8+ T-cell apoptosis during HIV-1 infection [220]. HIV-1 specific CD8+ T cells are highly susceptible to Fas mediated apoptosis, and may affect their ability to deal with HIV-1 infected cells [221]. CD8+ T cells are killed by HIV-1 infected macrophages [221]. HIV-1 infected macrophages produce TNF-α *in vivo* [222]. TNF-α is released as a soluble factor or expressed on the surface of macrophages in a membrane bound form that targets TNFR2 [223, 224]. TNFR-2 stimulation may trigger the apoptosis in CD8+ T cells [223]. Furthermore, TNF-α and TNF receptors increased during HIV-1 infection and inversely correlated with CD4+ T cell counts [225]. In fact, CD8+ T cells apoptosis in HIV-1 infection has been shown to result from interaction of the membrane bound form of TNF-α expressed on the surface of activated macrophages and TNFR-2 expressed on the surface of activated CD8+ T cells [69]. Following CXCR4 stimulation by HIV-1 gp120, both membrane bound TNF-α and TNFR-2 are upregulated on macrophages and CD8+ T cells, respectively [69]. TNFR-2 stimulation of T cells results in decreased intracellular levels of Bcl-$_{XL}$, a member of Bcl-2 family. Impaired induction of Bcl-$_{XL}$ has been observed in PBMCs isolated from HIV-1 infected patients [226].

HIV-1 infection of CD4+ T cells and macrophages results in the upregulation of FasL [30, 227]. The interaction of HIV-1 specific CD8+ T cells in the lymph nodes of patients with HIV-1 infected CD4+ T cells and macrophages could trigger Fas mediated apoptosis of HIV-1 specific CD8+ T cells [228]. Additionally, HIV-1 viral proteins such as Tat and Nef play an important role in bystander apoptosis. It has been demonstrated that Tat and Nef upregulate the expression of FasL and induces the apoptosis in CD8+ T cells [45, 229].

Thymic Dysfunction Reducing T-Cell Regeneration

The chronic immune activation during HIV-1 infection indirectly impairs the survival of naïve T cells by damaging the homeostatic mechanisms that maintain the normal populations of naïve T cells [230]. In the central lymphoid tissue such as thymus, the life of a T cell begins as a thymic progenitor cell [231]. In the thymus, CD3-CD4-CD8- triple negative thymocytes begins their differentiation and education through multiple interactions between thymocytes and the complex soluble and cellular component of the thymic microenvironment such as self-peptide-MHC-I as well as with IL-7 [232]. Eventually, thymocytes become CD4 or CD8 single positive naïve T cells and are exported to the periphery [231, 232].

HIV-1 infection also targets thymus. Examination of thymus in HIV-1 infection reveals a loss of lymphoid cells and a general destruction of thymic architecture [233-234]. HIV-1 replication in thymic microenvironment results in severe disruption of the normal processes of thymopoiesis [235]. Thymic function can be monitored by measuring TCR gene rearrangement excision circles (TREC). A reduction in TREC is observed in HIV-1 infection that reflects HIV induced impairment of thymic function [236]. Furthermore, thymus size is altered in HIV-1 infection and inversely correlated with viral load [237]. These changes in thymus can be partially reversed by antiretroviral therapy [236-238]. Experimental evidence of the infection of thymocytes with HIV has been carried out in thymocytes/TEC suspension culture. Thymocytes in these experimental culture system support high level of HIV-1 infection and replication [239, 240]. Furthermore, similar thymocyte dysfunction was also observed in macaques infected with SIV [241]. HIV-1 infects primarily CD4+ thymocytes in the thymus. However, other cell types such as dendritic cells can also be infected [233, 242]. Thymic DCs are important for thymopoiesis. These DCs are permissive to HIV-1 infection, resulting in apoptosis, thereby decreasing the number of available thymic DCs for selection which results in altered T-cell development [243]. Additionally, HIV-1 also infects and destroys the stromal cells. This destruction results in an altered thymic architecture that leads to impaired thymopoiesis [244, 245].

Most of HIV-1 replication occurs in the secondary lymphoid tissues, which triggers the chronic immune activation and associated tissue pathologies [246]. The secondary lymphoid tissues are organized to promote immune responses and maintain normal sized populations of T cells, B cells and antigen presenting cells (APC). T cells encounter antigen presented by antigen presenting cells (APC) in the parafollicular T cell zone, where naïve CD4+ and CD8+ T cells reside and gain excess to IL-7 and other factors required for their survival [247]. In the T cell zone, the fibroblastic reticular cell network (FRCn) gives the essential mechanical support to lymph nodes and is crucial for lymphocyte homeostasis within lymphoid tissues [248]. During the early phase of T-cell depletion by direct killing or other mechanisms such as AICD, the immune activation by HIV-1 elicits a T regulatory response that activates the TGFβ signaling pathway, thereby triggering the increased production of procollagen and chitinase 3-like-1 (CHI3L1) [18]. The collagen deposition interferes with the physical interaction between IL-7 bearing FRCn and naïve T cells. The survival of naïve T cell depends on IL-7, therefore, increased apoptosis by loss of access to and source of IL-7 results in depletion of naïve T cells [249, 250].

T-CELL SIGNALING AND FORMATION OF HIV-1 RESERVOIRS

HIV-1 replicates preferentially in activated CD4+ T cells [251, 252], but these cells generally survive for only few

days after infection [113]. When a CD4+ T cell encounters with an APC, it starts proliferation and enters into the cell cycle. After series of cell divisions, it gives rise to a clone of activated effector cells. Some of the cells revert back to a quiescent G0 state and persist as memory cells thus allowing rapid responses to future challenges with the same antigen [253]. Interestingly, HIV-1 gene expression is largely silenced as CD4+ T cells undergo in state of memory [254, 255]. Therefore, HIV-1 gene expression is heavily dependent on inducible host transcription factors (NF-κB and NFAT), which are excluded from the nuclei of resting cells [256, 257]. The ultimate result is a stably integrated but transcriptionally silent provirus in a memory T cells whose function is to survive for a longer period of time. Furthermore, if cells are activated by cytokines or other stimuli, they can start to produce virus. Moreover, in these cells, virus persists as integrated DNA that is unaffected by antiretroviral drugs [58].

Both naïve and resting CD4+ T cells provide very restricting environment for HIV-1 replication owing to low-level expression of CCR5 and low nucleotide pool and ATP level [258]. The reservoir of latently infected CD4+ T cells having replication competent HIV-1 genomes is established during primary infection [259]. These cells do not progress to complete viral replication unless they are activated [260], and their stability and long half-lives represent a major problem to HIV-1 eradication [261, 262]. It has been proposed that infected memory T cells can be expanded by homeostatic proliferation driven by IL-7 or low level proliferation driven by antigens [263]. Although resting CD4+ T cells or memory T cells cannot produce viable particles, HIV-1 Nef could increase T-cell activation and therefore facilitate HIV-1 replication in the same cell or in the surrounding cells [264]. After the activation of resting CD4+ T cells, the memory T cells could become infected during decay phase of activation, therefore allowing viral integration with no further progression to active replication [265].

CONCLUSION

T-cell receptor and signaling molecules are among the tools used by HIV-1 to fuel the progression of the disease. In HIV-1 infection, T-cell signaling events occurred in very well organized and coordinated manner. The survival and persistence of HIV-1 replication is dependent on numerous viral and non-viral factors. A number of T-cell signaling pathways is activated after binding of HIV-1 gp120 to chemokine receptor. These signaling events are very important for productive HIV-1 infection as these signaling pathways ultimately regulates several cellular functions such as cytoskeletal rearrangement, cell survival and differentiation, and activation of several cellular transcription factors. The T cells are essential in the formation of viral reservoirs and play critical role in HIV-1 disease progression. T-cell activation renders both naïve and memory-resting T cells susceptible to infection. Several HIV-1 proteins modulate the T-cell signaling in infected and bystander T cells thereby facilitating disease progression. The infection of resting CD4+ T cells is usually inefficient. To overcome the cellular barriers, HIV relies on its proteins and their binding to cellular partners. Nef and Tat are expressed very early after infection before the integration of HIV-1 genome and activate transcription factors such as NF-kB that favor cell survival. Then Vpr and Tat activate HIV transcription to complete HIV-1 replicative cycle. At later stages of disease, the combined effect of the viral proteins and perturbation of cytokine signaling fuels HIV-1 pathogenesis. The deleterious effects of immune activation by HIV-1 infection on T-cell signaling and homeostasis could be specifically targeted with new immune-based therapies in addition to standard highly active antiretroviral therapy (HAART). A better understanding of the dynamic interaction between HIV-1 and the host immune system, more specifically T-cell signaling, may lead to the development of vaccine and antiviral strategies that can limit HIV-1 pathogenesis.

CONFLICT OF INTEREST

GH is the member of the editorial board of the "*The Open Virology Journal*". The other authors confirm that this article content has no other conflict of interest.

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